IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant	:	Chaikof et al.	
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For	:	PLASTIC AND ELASTIC PROTEIN COPOLYMERS	
Docket No.	:	133-02	
Customer No.:		23713	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.131

OF PRIOR INVENTION IN THE UNITED STATES OR IN A NAFTA OR WTO MEMBER COUNTRY TO OVERCOME CITED PATENT OR PUBLICATION

BY VINCENT P. CONTICELLO

Sir:

Vincent P. Conticello hereby declares as follows:

1. Vincent P. Conticello, Ph.D. is an inventor of the above-identified patent application.
2. I am a Professor of Chemistry in the Department of Chemistry at Emory University, Atlanta, Georgia, United States of America.
3. I have experience and expertise in the technical field of biomaterials and biological polymer synthesis including for protein-based polymers.

4. I understand that in the above-referenced patent application, the following references have been cited as the basis of a prior art rejection under 35 U.S.C. 102(a) in an Office Action dated May 8, 2009:

   a. Wright and Conticello (Adv. Drug Deliv. Rev., Oct. 2002, 54: 1057-73) titled "Self-assembly of block copolymers derived from elastin-mimetic polypeptide sequences." (hereinafter "*Wright and Conticello*").

   b. Wright, McMillan, Cooper, Apkarian and Conticello (Adv. Funct. Materials, Feb. 2002, 12:149-154) titled "Thermoplastic elastomer hydrogels via self-assembly of an elastin-mimetic triblock polypeptide." (hereinafter "*Wright et al.*")

5. I am an author of the *Wright and Conticello* reference cited in the Office Action dated May 8, 2009.

6. I believe that the invention in the above-referenced application relevant to the *Wright and Conticello* reference was made at least as early as the 102(a) date of the cited reference.

7. I understand the Office is using Table 2 of *Wright and Conticello* to reject various claims in the above-referenced application under 35 U.S.C. 102(a). As an author of the reference, I supplied or caused to be supplied to the journal Advanced Drug Delivery Reviews, the subject matter that is being relied upon by the Office to reject the various claims, including the sequences contained in Table 2, on a date that is earlier than the 102(a) date. This is reflected on the face of the Wright and Conticello reference (p.1057) (provided as Exhibit A), where the accepted date of the reference is listed as July 1, 2002, which is earlier than the Oct. 2002 102(a) date of *Wright and Conticello*.

8. I am an author of the *Wright et al.* reference cited in the Office Action dated May 8, 2009.

9. I believe that the invention in the above-referenced application relevant to the *Wright et al.* reference was made at least as early as the 102(a) date of the cited reference.

10. I understand the Office is using Scheme 1 on p.149 of *Wright et al.* to reject various claims in the above-referenced application under 35 U.S.C. 102(a). As an author of the reference, I supplied or caused to be supplied to the journal Advanced Functional Materials, the subject matter that is being relied upon by the Office to reject the various claims, including the sequences contained in Scheme 1, on a date that is earlier than the 102(a) date. This is reflected on p.154 of the Wright et al. reference (provided as Exhibit B), where the received date of the reference is listed as December 17, 2001, which is earlier than the Feb. 2002 102(a) date of *Wright et al.*

11. In support of this Declaration, I have provided as Exhibits true copies of relevant pages of each reference that indicate on their face that I supplied or caused to be supplied the information being used to reject the claims before the relevant 102(a) dates of each of *Wright and Conticello* and *Wright et al.* I believe these Exhibits, which were submitted by me personally or on my behalf and of which I have personal knowledge, demonstrates the existence of the invention considered relevant to *Wright et al.* and *Wright and Conticello* on or before the respective cited references' 102(a) dates.

12. For any of the content of the cited references which is related to the invention claimed in the instant patent application, the invention or portion thereof was made at least as early as prior to the dates of the two cited references.

13. All statements made herein of my own knowledge are true, and all statements made on information and belief are believed to be true; these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under 18 United States Code §1001

and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

BY: Vincent P. Conticello

Vincent P. Conticello

November 5, 2009
Date

GREENLEE, WINNER AND SULLIVAN, P.C.
4875 Pearl East Circle, Suite 200
Boulder, CO 80301
Telephone: (303) 499-8080
Facsimile: (303) 499-8089
Attorney Docket No. 133-02

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Self-assembly of block copolymers derived from elastin-mimetic polypeptide sequences

Elizabeth R. Wright, Vincent P. Conticello*

Department of Chemistry, Emory University, 1515 Pierce Drive, Atlanta, GA 30322, USA

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Abstract

Protein polymers derived from elastin-mimetic peptide sequences can be synthesized with near-absolute control of macromolecular architecture using genetic engineering techniques. Elastin-mimetic diblock and triblock copolymers have been prepared using this approach in which the individual elastin blocks display different phase behavior in aqueous solution. The selective collapse of the more hydrophobic blocks above the lower critical solution temperature was employed to drive the thermo-reversible self-assembly of elastin-mimetic diblock and triblock copolymer into protein-based nanoparticles and nano-textured hydrogels, respectively. These materials display considerable promise as biomaterials for applications in drug delivery and soft tissue augmentation.

Keywords: Elastin; Biomaterials; Protein polymer; Nanoparticle; Hydrogel; Genetic engineering

Contents

1. Introduction

The creation of novel, biocompatible reagents for encapsulation and release of medically important molecules such as pharmaceuticals and image contrast agents represents an important goal of biomedical research. Microstructured materials derived from self-assembly of synthetic amphiphilic block copolymers are being seriously scrutinized as alternatives to small molecule surfactants (micelle-forming detergents) for the controlled delivery and release of diffusible substrates (small molecules, oligonucleotides, and proteins) in medical applications [1,2].

*Corresponding author.
E-mail address: vcontic@emory.edu (V.P. Conticello).

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ADVANCED FUNCTIONAL MATERIALS

reduce signal due to the residual protons of water in the ^{1}H NMR of aqueous solutions. The HMQC NMR experiments were acquired at 4 °C and 25 °C with a 90° pulse of 8 μs on the proton (sweep width 5500.2 Hz) and a 90° pulse of 12 μs on the carbon. The data matrix contained 256 t_1 increments (sweep width in F_1 (carbon), 33 999.2 Hz) at 96 scans per increment. The NMR data were processed using the program NutsPro from Acorn NMR, Inc. (Livermore, CA).

Rheology Measurements: A parallel plate arrangement was employed in which the plate had a diameter of 24.94 mm, a concentricity of 6 μm, and a parallelity of 3 μm. Samples of the polypeptides were prepared as 20–25 wt.-% solutions of protein in sterile, deionized H_2O and were equilibrated at 4 °C prior to the measurements. Approximately 300 μL of the sample was applied to the bottom plate of the rheometer. The top plate was lowered to a distance of 0.5 mm. Silicon oil was applied around the plate and sample's circumference in order to prevent evaporative loss of solvent from the sample during the measurements. Three different tests were performed sequentially as follows. Temperature sweeps were recorded from 3 °C to 25 °C over a period of eleven minutes in duration (0.5 min at each temperature) with a strain of 1 % at an angular frequency of 10 rad/s. Replicate measurements were recorded under identical conditions to ensure reproducibility of the temperature response. Amplitude sweeps of thirty measurements were taken with strains (γ) ranging from 0.1 to 100 % at an angular frequency (ω) of 10 rad/s. Frequency sweeps of twenty-one measurements at 1 % strain were recorded at 25 °C with the frequency ranging from 0.1 to 10 Hz was taken. The frequency sweep was repeated seven times before the plate and sample was cooled to 3 °C in order to duplicate all the experiments.

Cryo HRSEM Experiments: Approximately 5 to 10 μL of the solutions were pipetted into 3 mm gold planchets (Balzers BU 012 130T) that had been pre-equilibrated to 4 °C in an isothermal environmental cooler. The temperature of the cooler was raised to approximately 25 °C and allowed to stabilize for 10 min. The solidified samples were plunged into liquid ethane at its melting point (–183 °C) and the vitrified samples were stored in liquid nitrogen (LN_2). A sample was transferred to and mounted on the precooled (ca. –170 °C) Oxford CT-3500 cryo-stage held in the cryo-preparation chamber. The specimen was fractured with a prechilled blade and washed with LN_2. The shutters on the stage were closed to minimize frost contamination and the cryo-stage was transferred to the Denton DV-602 Cr coater. At this point, if the samples were to be etched in order to remove excess water (vitreous ice), the stage was allowed to equilibrate in a vacuum of $\sim 10^{-7}$ torr. Once this occurred, the shutters were opened and the stage was brought to a temperature between –105 °C and –99 °C for varying time intervals. The stage shutters were closed and the stage was returned to –170 °C. A monatomic (1 nm) film of chromium was sputter coated onto the specimen, the stage shutters were closed and the stage was transferred to the upper stage of the DS-130F field-emission SEM operated at 25 kV. During the imaging process, specimen temperature was maintained at –115 °C. Images were digitally collected (5 Mbytes) in 16 s in order to reduce radiation damage.

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[15] Copolymer samples for DSC (ca. 1 mg/mL) were dissolved at 4 °C in either 40 mM NaOH or 40 mM ethanoic (acetic) acid and thermal transition data recorded over a temperature range from 4 °C to 60 °C using a Microcal VP-DSC instrument at a scan rate of 1 °C/min. Reversibility was tested by cooling and re-scan of samples in situ. Data were processed and analyzed using Microcal ORIGIN software.
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[20] Rheology measurements were performed on a Paar Physica MCR 300 instrument. See Experimental section for details.
[21] Cryo-HRSEM measurements were performed on copolymers **1** and **2**, which were prepared as 20 to 25 % protein solutions in deionized H_2O at 4 °C. A short description is given in the Experimental section.
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[24] Abbreviations for amino acids: A, alanine; E, glutamic acid; G, glycine; I, isoleucine; P, proline; and V, valine.

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